UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/932,254	08/16/2001	Lu Liu	0162.210US	3106

27310 7590 07/27/2005

PIONEER HI-BRED INTERNATIONAL, INC.
7250 N.W. 62ND AVENUE
P.O. BOX 552
JOHNSTON, IA 50131-0552

EXAMINER	
VOGEL, NANCY S	

ART UNIT	PAPER NUMBER
1636	

DATE MAILED: 07/27/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/932,254	LIU ET AL.	
	Examiner	Art Unit	
	Nancy T. Vogel	1636	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-105* is/are pending in the application.

4a) Of the above claim(s) *7,9,10,21-31,40,42-79,81 and 96-104* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-6,8,11-20,32-39,41,80 and 82-95* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *16 August 2001* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *see next page*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

Attachment(s)
3) 12/12/01, 3/14/02, 8/8/02, 9/17/02, 3/26/03.

DETAILED ACTION

Election/Restrictions

Applicant's election of Group I, and further the species of B, diaminobutyric acid aminotransferase, a diaminobutyric acid acetyltransferase, or a ectoine synthase in the reply filed on 5/31/05 is acknowledged. Because applicant did not distinctly and specifically point out the supposed errors in the restriction requirement, the election has been treated as an election without traverse (MPEP § 818.03(a)).

Claims 1-6, 8, 11-20, 32-39, 41, 80, 82-95, and 105 read on the elected species.

Claims 7, 9, 10, 21-31, 40, 42-79, 81, and 96-104, are withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected species, there being no allowable generic or linking claim. Election was made **without** traverse in the reply filed on 5/31/05.

Claim Rejections - 35 USC § 102

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 1, 2, 12, 13 and 19 are rejected under 35 U.S.C. 102(b) as being anticipated by Affhotler et al. (WO 98/36080) (cited as CF on Information Disclosure Statement filed 12/12/01).

Affhotler et al. disclose a method of producing a modified gene fusion construct, comprising cojoining two heterologous nucleic acid sequences, wherein each sequence encodes one or more enzymatic domains, and wherein at least one of the two or more heterologous nucleic acid sequences is modified (see page 29, line 30 – page 30, line

16). The two heterologous nucleic acid sequences encode thioredoxin and dehalogenase. The nucleic acid sequence encoding the dehalogenase is modified (see page 30, lines 8-16). The two sequences are connected by one or more nucleotide linker sequences which comprise a restriction enzyme recognition site, and therefore is estimated to be between 3 and 200 nucleotides in length (see page 30, lines 8-16 and Fig. 8). The modified gene fusion construct comprises one or more transcription regulatory sequences (see page 30 lines 2-7).

Claims 1, 2, 4, 11-14, 16-20, are rejected under 35 U.S.C. 102(b) as being anticipated by Gilbert et al. (WO 99/31224-A, listed as Cite. No. 1 on Information Disclosure Statement submitted 3/26/03).

Gilbert et al. disclose a method of producing a modified gene fusion construct, comprising cojoining two or more heterologous nucleic acid sequences wherein each sequence encodes one or more enzymatic domains, and wherein at least one other two or more heterologous nucleic acid sequences is modified. The two nucleic acid sequences encode such enzymes as glycosyltransferase or sialtransferase and an accessory enzyme such as glucokinase or sialylsynthetase (see page 11 line 27 – page 12 line 7, see pages 39-40 line 16). The nucleic acids are modified, since a region encoding a His tag is added, restriction sites are added, a peptide linker, and a c-Myc epitope tag is added (page 43, lines 10-28). The modified heterologous genes may be considered to be linked directly to each other, since the modified heterologous gene can be considered to encompass the original gene sequence with the peptide linker. The

reference discloses a peptide linker that comprises 4, 8 or 9 amino acids (page 43, lines 19-26), which thus falls within the limits of 3-300 or 12-90 nucleotides. The reference teaches that the linker may be a poly-glycine peptide (page 26, lines 20-30). The reference discloses that the linker may comprise a cleavage site (page 34, lines 4-10). The reference discloses the method in which the fusion construct further comprises one or more transcription regulatory sequences (page 40, lines 12-22). The transcription regulatory sequence may be from plant (see pages 28, line 26 – page 29, line 20).

Claims 1-5 are rejected under 35 U.S.C. 102(b) as being anticipated by Nixon et al. (Tibtech 1998, 16:258-264, cited on Information Disclosure Statement filed 3/14/02).

Nixon et al disclose a method of producing a modified gene fusion construct comprising cojoining two or more heterologous nucleic acid sequences, each of which encodes one or more enzymatic domains, and at least one of which is modified (see Figure 1; see paragraph bridging pages 258 and 259; see page 261, first column, line 1 – page 262, second column, last paragraph). Nixon et al. discloses the method of producing a hybrid enzyme by the stochastic approach of DNA shuffling (see page 263, second column; third complete paragraph)

Claims 1, 4, 11, 19, 20, 32-38, 80, 82, 83, 86, 94 and 95 are rejected under 35 U.S.C. 102(e) as being anticipated by Coruzzi et al. (US Patent 6,864,405).

The reference discloses a method of producing a modified gene fusion construct comprising cojoining two or more heterologous nucleic acid sequences each encoding

an enzymatic domain, wherein at least one is from a plant, and the regulatory sequence is from a plant. The enzymatic domains may be from the same or different organisms and may include modifications in such residues as those involved in substrate binding and/or catalysis (see column 6, lines 24-48, column 12, lines 24-54). The reference discloses that one of the enzymatic domains may be from a plant, and that a plant promoter may be used (see column 13 line 65 – column 14, line 15). The reference discloses the direct linkage of the domains (see Fig. 2).

Claims 1-5, 11-14, 16, 19 and 20 are rejected under 35 U.S.C. 102(a) as being anticipated by Peoples (WO 00 06747, cited on Information Disclosure Statement filed 3/14/02).

Peoples et al. disclose a method of making a modified gene fusion construct comprising cojoining two or more heterologous nucleic acid sequences, wherein each encodes one or more enzymatic domains, and at least one is modified (see page 4, lines 14-40). The reference discloses that linkers may or may not be added and discloses that linkers of any appropriate length, such as 6 or 15 nucleotides may be used (see page 4-5 and 20). The reference discloses that the nucleic acid sequences may be those that participate in the same metabolic pathway (page 7 lines 10-21). The reference discloses that the two enzymes may be modified by gene shuffling (page 7 lines 22-28). The reference discloses the method in which the gene fusion construct comprises a transcriptional regulatory sequence, which may be from a plant (page 11-12).

Claims 1, 2, 12-15, 19, 20, 32-35, 38, 80, 83, 87-90, 94, 95 are rejected under 35 U.S.C. 102(b) as being anticipated by Rose et al. (US Patent 5,861,277).

Rose et al. disclose a method of producing a gene fusion construct comprising cojoining two or more nucleic acid sequences encoding at least two enzymatic domains, wherein at least on the nucleic acid sequences is from a plant, and the other is from a prokaryote (see Fig. 1, see col. 13, lines 3-24). The nucleic acid sequences are modified since they contain deletions. They are connected by a linker which comprises one or more intron sequences (see Fig. 1). The construct comprises a plant transcription regulatory sequence, ie the PAT1 promoter. The first intron contains 110 nucleotides (see SEQ ID NO:3).

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was

not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claim 6 is rejected under 35 U.S.C. 103(a) as being unpatentable over Peoples et al. (WO 00 06747) in view of Minshull et al. (WO 97/35966) (cited as BS on Information disclosure Statement submitted 12/12/01).

Peoples et al. is cited essentially for the reasons set forth above.

The difference between the reference and the instant claim is that the shuffling comprises recursive sequence recombination.

However, Minshull et al. disclose the method of shuffling which is recursive sequence recombination, and its usefulness for modifying or evolving the structure of genes, including multiple genes, and including genes encoding enzymes, in order to produce molecules with improved and changed properties (see page 3, lines 4-15 for instance). The reference discloses that this technique allows the recombination of a large number of mutations in a minimum number of selection cycles (page 3, line 12-15).

It would have been obvious to one of ordinary skill in the art, to have utilized such well known techniques of gene shuffling as recursive sequence recombination, as taught by Minshull et al., in the method of producing a mofified gene fusion construct as taught by Peoples et al., since both references disclose the desirability of altering a gene sequence of interest in order to produce an improved, or altered enzyme product,

by the general method of gene shuffling. One would have been motivated to do so by the known advantage, disclosed by Minshull et al., of the ability to recombine a large number of mutations in a minimum number of selection cycles.

Claim 8 is rejected under 35 U.S.C. 103(a) as being unpatentable over Bulow et al. (Trends in Biotech. 9:226-231, 1991) in view of Goller et al. (FEMS Micro. Lett. 161 293-300, 1998), Lous et al. (Microbiology, 143:1141-1149, 1997), Nakayama et al. (Plant Physiol. 122, 1239-1247, 2000).

Bulow et al. teach fusion enzymes expressed in Escherichia coli that catalyze sequential catalytic steps, namely a beta-galactosidase-galactokinase protein fusion that catalyzes 1) the hydrolysis of lactose to glucose and galactose, followed by 2) phosphorylation to galactose-l- phosphate (page 228, Fig. 3). Bulow teach that "When corrected for the increase in molecular weight caused by the fusion, the specific activities correspond to 50-100% of those for the native enzymes" (page 230, column 1). "Furthermore, only modest changes in Km, pH-optima, thermo stability. . . . . have been observed" (page 230, column 1). Bulow et al. also teach advantages of fusion enzymes that catalyze sequential steps, such as facilitated purification, favorable enzyme kinetics, and proximity effects whereby an intermediate product can be transferred efficiently to the desired second enzyme, instead of a competing enzyme (page 226). Bulow et al. further teach that "fairly short linkers (two to ten amino acid residues) are optimal - longer linkers are often prone to proteolytic degradation in vivo and the final yield of recombinant enzymes can be reduced severely" (page 230,

column 1). Bulow et al. also teach that "the order of the genes, which determines which enzyme forms the N-terminus of the final gene product, is chosen arbitrarily" and that "the reason this design principle can, in general be applied successfully is that the N- and C-termini of the proteins are charged and therefore usually located on the surface. The native tertiary structure of the constituent proteins thus remains almost intact in the recombinant enzyme" (page 227, column 1). Bulow et al. do not explicitly teach a method of producing a modified gene fusion construct comprising cojoining two or more heterologous nucleic acid sequences encoding enzymatic domains selected from the group consisting of diaminobutyric acid aminotransferase, diaminobutyric acid acetyltransferase, and ectoine synthase, from either prokaryotic or eukaryotic sources, including plants.

Goller et al., Louis et al. or Nakayama et al. each teach the cloning, nucleotide and amino acid sequences of genes of the biosynthetic pathway of ectoine, ie diaminobutyric acid acetyltransferase, diaminobutyric aminotransferase, and ectoine synthase from the prokaryotes H. elongate and the M. halophilus.

It would have been obvious to one of ordinary skill in the art to combine the teachings of Bulow et al., and Goller et al. Louis et al. or Nakayama et al., for a method of producing a modified gene fusion construct, in which the genes encoding the enzymes involved in the ectoine synthesis pathway, are cojoined to produce a modified gene fusion construct, since all of the references are concerned with the synthesis of biologically useful products using the cloned genes encoding enzymes involved in the biosynthetic pathway of said products. One would have been motivated to have used

the method disclosed by Bulow et al. for the production of a gene fusion construct comprising the biosynthetic enzyme encoding genes disclosed by Goller et al, Louis et al. or Nakayama et al., since Goller et al., Louis et al., and Nakayama et al. each disclose the usefulness of ectoine in osmoadaptation, and further that the expression of the enzymes encoded by the disclosed genes is desirable in heterologous cells such as plants, and since Bulow et al. disclose the desirability of expressing any biosynthetic enzyme encoding genes involved in a single pathway, together in a gene fusion construct, in order to obtain the benefit of such as facilitated purification, favorable enzyme kinetics, and proximity effects whereby an intermediate product can be transferred efficiently to the desired second enzyme, instead of a competing enzyme. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claim 15 is rejected under 35 U.S.C. 103(a) as being unpatentable over Gilbert et al. (WO 99/31224) in view of Rose et al. (US Patent 5,861,277).

Gilbert et al. is cited essentially for the reasons set forth above.

The difference between the reference and the instant claim is that the linker between the two heterologous nucleic acids encoding enzymatic domains is an intron.

However, Rose et al. disclose methods of making gene fusions in which an intron is placed between two nucleic acid sequences encoding enzymatic domains (see col.

13, lines 3-25). Rose disclose that such structures increase expression levels (col. 13, lines 3-10).

It would have been obvious to one of ordinary skill in the art to have modified the method of producing a gene fusion construct comprising two heterologous nucleic acids connected by a linker, by using a linker comprising an intron, as disclosed by Rose et al., since both the references disclose methods of producing constructs comprising nucleic acids which link two heterologous nucleic acids of interest. One would have been motivated to do so by the teaching of Rose et al. that the use of an intron in linker sequence results in the higher levels of expression. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claims 84 and 85 are rejected under 35 U.S.C. 102(e) as being anticipated by Coruzzi et al. (US Patent 6,864,405) or Rose et al. (US Patent 5,861,277) in view of Minshull et al. (WO 97/35966) (cited as BS on Information disclosure Statement submitted 12/12/01).

Coruzzi et al. and Rose et al. are cited essentially for the reasons set forth above.

The difference between the reference and the instant claim is that the shuffling comprises recursive sequence recombination.

However, Minshull et al. disclose the method of shuffling which is recursive sequence recombination, and its usefulness for modifying or evolving the structure of genes, including multiple genes, and including genes encoding enzymes, in order to produce molecules with improved and changed properties (see page 3, lines 4-15 for instance). The reference discloses that this technique allows the recombination of a large number of mutations in a minimum number of selection cycles (page 3, line 12-15).

It would have been obvious to one of ordinary skill in the art, to have utilized such well known techniques of gene shuffling as recursive sequence recombination, as taught by Minshull et al., in the method of producing a modified gene fusion construct as taught by Coruzzi et al. or Rose et al et al., since both references disclose the desirability of altering a gene sequence of interest in order to produce an improved, or altered enzyme product. One would have been motivated to do so by the known advantage, disclosed by Minshull et al., of the ability to recombine a large number of mutations in a minimum number of selection cycles when using the method of recursive sequence recombination generally to alter the structure and function of any protein or enzyme of interest in order to obtain the benefit of improved function. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claims 92 and 93 are rejected under 35 U.S.C. 103(a) as being unpatentable over Coruzzi et al. . (US Patent 6,864,405) or Rose et al. (US Patent 5,861,277) in view of Gilbert et al. (WO 99/31224-A).

Coruzzi et al. and Rose et al. are cited essentially for the reasons set forth above in the rejections under 35 USC 102.

The difference between the references and the instant claims is that the peptide linker contains a cleavable site, or the peptide linker comprises at least 80% glycine residues.

However, Gilbert et al. teach a method of making a gene fusion construct, in which two heterologous genes are linked by a peptide linker that may be poly-glycine, or may contain a cleavable site (see page 26, lines 20-30 and page 34, lines 4-10). Gilbert et al. teaches that poly-glycine linkers provide flexibility (page 26-27).

It would have obvious to one of ordinary skill in the art to have combined the teachings of Course et al., Rose and Gilbert et al. to produce a method of making , since all of,the references teach methods of making a modified gene fusion construct in which two heterologous nucleic acids encode at least two enzymatic domains. One would have been motivated to have included such well known peptide linker regions as those including cleavable sites, or those comprising poly-glycine, in order to obtain the benefits of obtaining separate enzymes when desired, and to obtain the known structural properties resulting from peptides rich in glycine, which include flexibility, as taught by Gilbert. Based upon the teachings of the cited references, the high skill of

one of ordinary skill in the art, and absent evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 8, 41 and 105 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claims 8, 41, and 105 are directed to a method of producing a modified gene fusion construct which comprises two or more heterologous nucleic acid sequences encoding enzymatic domains selected from the group consisting of a genus of diaminobutyric acid aminotransferases, a genus of diaminobutyric acid acetyltransferases, and a genus of ectoine synthases which have not been adequately described in the specification. The specification teaches the structures of only species of these enzymes which are isolated from the eubacteria H. elongate, and M. halophilus (page 60). Moreover, the specification fails to describe any other representative species by any identifying characteristics or properties other than the functionality of being a diaminobutyric acid aminotransferase, a diaminobutyric acid acetyltransferase,

or a ectoine synthase. Given this lack of description of representative species encompassed by the genus of the claim, the specification fails to sufficiently describe the claimed invention in such full, clear, concise and exact terms that a skilled artisan would recognize that applicants were in possession of the claimed invention.

Conclusion

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Nancy T. Vogel whose telephone number is (571) 272-0780. The examiner can normally be reached on 7:00 - 3:30, Monday - Friday.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Irem Yucel, Ph.D. can be reached on (571) 272-0781. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

N. T. Vogel

NANCY VOGEL, PH.D.
PATENT EXAMINER

N. Vogel
Patent Examiner